invitrogen™

pEF4/*myc*-His A, B, and C

Catalog no. V942-20

Rev. date: 30 December 2010

Manual part no. 25-0239

MAN0000081

User Manual

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Kit Contents and Storage

Shipping and Storage

pEF4/*myc*-His vectors are shipped on wet ice. Upon receipt, store vectors at -20°C.

Kit Contents

All vectors are supplied as detailed below. **Store the vectors at –20°C.**

Vector	Composition	Amount
pEF4/*myc*-His A, B, and C	40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg
pEF4/*myc*-His/*lacZ*	40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0	20 µg

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Introduction

Product Overview

Description of the System

pEF4/*myc*-His A, B, and C are 5.9 kb vectors designed for overproduction of recombinant proteins in mammalian cell lines. Features of the vectors allow purification and detection of expressed proteins (see pages12–13 for more information). High-level stable and transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human elongation factor 1α-subunit (hEF-1α) promoter for high-level expression across a broad range of species and cell types (Goldman *et al.*, 1996) (Mizushima and Nagata, 1990) (see page 11 for more information).
- Three reading frames to facilitate in-frame cloning with a C-terminal peptide encoding the *myc* epitope and a polyhistidine (6×His) metal-binding tag.
- Zeocin™ resistance gene for selection of stable cell lines* (Mulsant *et al.*, 1988) (see page 15 for more information).
- Episomal replication in cell lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS7).

The control plasmid, pEF4/*myc*-His/*lac*Z is included for use as a positive control for transfection, expression, and detection in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pEF4/*myc*-His.

- Consult the multiple cloning sites described on pages 3–5 to determine which vector (A, B, or C) should be used to clone your gene in frame with the C-terminal *myc* epitope and the polyhistidine tag.
- Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on 50 to 100 μg/mL ampicillin or 25 to 50 μg/mL Zeocin™ in Low Salt LB. For more information, see page 17.
- Analyze your transformants for the presence of insert by restriction digestion.
- Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is in frame with the C-terminal peptide.
- Transfect your construct into the cell line of choice using your own method of transfection. Generate a stable cell line, if desired.
- Test for expression of your recombinant gene by western blot analysis or functional assay. For antibodies to the *myc* epitope or the C-terminal polyhistidine tag, see page 18.
- To purify your recombinant protein, you may use metal-chelating resin such as ProBond™. ProBond™ resin is available separately (see page 18 for ordering information).

Methods

Cloning into pEF4/*myc*-His A, B, and C

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, purification of single-stranded DNA, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Strain

Many *E. coli* strains are suitable for the growth of this vector including TOP10F´, DH5αF´, JM109, and INVαF´. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10F´ is available as chemically competent or electrocompetent cells from Invitrogen.

Transformation Method

You may use any method of your choice for transformation. Chemical transformation is the most convenient for most researchers. Electroporation is the most efficient and the method of choice for large plasmids.

Maintaining pEF4/*myc*-His

To propagate and maintain the pEF4/*myc*-His vectors, use a small amount of the supplied 0.5 µg/µL stock solution in TE, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10F´, DH5α, JM109, or equivalent. Select transformants on LB plates containing 50–100 µg/mL ampicillin or 25 to 50 µg/mL Zeocin™ in Low Salt LB. Be sure to prepare a glycerol stock of each plasmid for long term storage (see page 6).

Cloning Considerations

Your insert should contain a Kozak consensus sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak 1990). An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position –3 and the G at position +4 (shown in bold) illustrates the most commonly occurring sequence with strong consensus. Replacing one of the two bases at these positions provides moderate consensus, while having neither results in weak consensus. The ATG initiation codon is shown underlined.

(**G/A**)NNATG**G**

If you wish to express your protein WITHOUT the C-terminal peptide, be sure to include a stop codon.

Continued on next page

Cloning into pEF4/*myc*-His A, B, and C, Continued

Multiple Cloning Site of Version A

Below is the multiple cloning site for pEF4/*myc*-His A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. **Note that there is a stop codon between the *Spe* I site and the *BstX* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. For more information on the hEF-1α promoter, see page 11. The vector sequence of pEF4/*myc*-His A is available for downloading from www.invitrogen.com or from **Technical Support** (see page 19).

```
                                                                    3´end of hEF-1α Intron 1
1581  GTTTGGATCT TGGTTCATTC TCAAGCCTCA GACAGTGGTT CAAAGTTTTT TTCTTCCATT TCAGGTGTCG TGAGGAATTA
                                                                    5´ end of hEF-1α Exon 2

                    T7 promoter/priming site                                  Acc65 I  Kpn I          BamH I    Spe I
1661  GCTTGGTACT AATACGACTC ACTATAGGGA GACCCAAGCT GGCTAGT TAA GCT TGG TAC CGA GCT CGG ATC CAC
                                                        *** Ala Trp Tyr Arg Ala Arg Ile His

                      BstX I*  EcoR I              EcoR V          BstX I*  Not I              Xba I
1735  TAG TCC AGT GTG GTG GAA TTC TGC AGA TAT CCA GCA CAG TGG CGG CCG CTC GAG TCT AGA GGG CCC
      *** Ser Ser Val Val Glu Phe Cys Arg Tyr Pro Ala Gln Trp Arg Pro Leu Glu Ser Arg Gly Pro

      BstB I  |------------ myc epitope ------------|                          |---- Polyhistidine tag ----|
1801  TTC GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT ATG CAT ACC GGT CAT CAT CAC CAT CAC CAT
      Phe Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Met His Thr Gly His His His His His His

            Pme I                    BGH reverse priming site
1867  TGA G TTTAAACCCG CTGATCAGCC TCGACTGTGC CTTCTAGTTG CCAGCCATCT GTTGTTTGCC CCTCCCCCGT
      ***

1941  GCCTTCCTTG ACCCTGGAAG GTGCCACTCC CACTGTCCTT TCCTAATAAA ATGAGGAAAT TGCATCGCAT TGTCTGAGTA
```

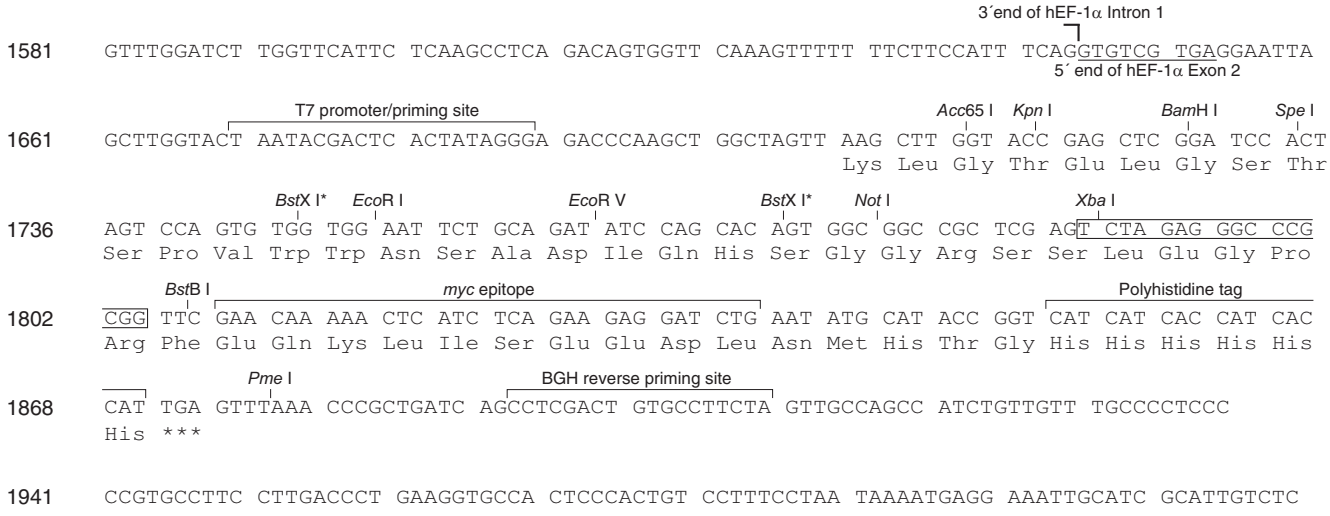
*Note that there are two *BstX* I sites in the polylinker.

Continued on next page

Cloning into pEF4/*myc*-His A, B, and C, Continued

Multiple Cloning Site of Version B

Below is the multiple cloning site for pEF4/*myc*-His B. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. For more information on the hEF-1α promoter, see page 11. The vector sequence of pEF4/*myc*-His B is available for downloading from www.invitrogen.com or from **Technical Support** (see page 19).



*Note that there are two *BstX* I sites in the polylinker.

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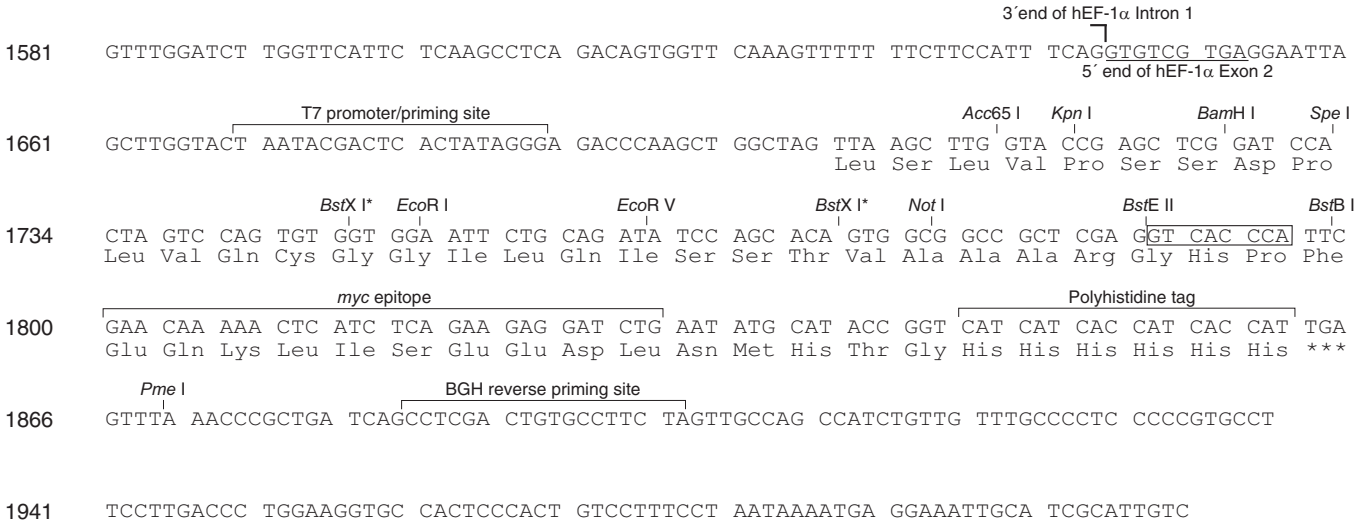
Cloning into pEF4/*myc*-His A, B, and C, Continued

Multiple Cloning Site of Version C

Below is the multiple cloning site for pEF4/*myc*-His C. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. For more information on the hEF-1α promoter, see page 11. The vector sequence of pEF4/*myc*-His C is available for downloading from www.invitrogen.com or from **Technical Support** (see page 19).



*Note that there are two *BstX* I sites in the polylinker.

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Cloning into pEF4/*myc*-His A, B, and C, Continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (*e.g.,* TOP10F´, DH5α) and select on LB plates containing 50–100 µg/mL ampicillin or 25–50 µg/mL Zeocin™ in Low Salt LB (see below). Select 10–20 clones and analyze for the presence and orientation of your insert.

Important

Any *E. coli* strain that contains the complete Tn*5* transposable element (*i.e.* DH5αF´IQ) encodes the *ble* (bleomycin resistance gene). These strains will confer resistance to Zeocin™. For the most efficient selection, we highly recommend that you choose an *E. coli* strain that does not contain the Tn*5* gene (*i.e.* TOP10, DH5α, DH10, etc.).

We recommend that you sequence your construct with the T7 Forward and BGH Reverse primers to confirm that your gene is fused in frame with the *myc* epitope and the C-terminal polyhistidine tag. Refer to the diagrams on pages 3-5 for sequences and location of primer binding sites.

Preparing a Glycerol Stock

Once you have identified the correct clone, be sure to purify the colony and make a glycerol stock for long-term storage. It is also a good idea to keep a DNA stock of your plasmid at –20°C.

1. Streak the original colony out on an LB plate containing 50 µg/mL ampicillin or 25 µg/mL Zeocin™ in Low Salt LB. Incubate the plate at 37°C overnight.
2. Isolate a single colony and inoculate into 1–2 mL of LB containing 50 µg/mL ampicillin or 25 µg/mL Zeocin™.
3. Grow the culture to mid-log phase (OD_{600} = 0.5–0.7).
4. Mix 0.85 mL of culture with 0.15 mL of sterile glycerol and transfer to a cryovial.
5. Store at –80°C.

Transfection and Analysis

Introduction

Once you have confirmed that your construct is in the correct orientation and fused in frame to the C-terminal peptide, you are ready to transfect your cell line of choice. We recommend that you include the positive control vector and a mock transfection to evaluate your results.

Plasmid Preparation

Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HiPure Miniprep Kit or the PureLink™ HiPure Midiprep Kit (see page 18).

Methods of Transfection

For established cell lines (*e.g.* HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. We recommend that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology*.

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). Invitrogen offers the Lipofectamine™ 2000 Reagent for mammalian transfection (see page 18 for ordering information).

Positive Control

pEF4/*myc*-His/*lac*Z is provided as a positive control vector for mammalian transfection and expression (see page 14), and may be used to optimize transfection conditions for your cell line. The gene encoding β-galactosidase is expressed in mammalian cells under the control of the hEF-1α promoter. A successful transfection will result in β-galactosidase expression that can be easily assayed (see below).

Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. Invitrogen offers the β-Gal Assay Kit and the β-Gal Staining Kit for fast and easy detection of β-galactosidase expression (see page 18 for ordering information).

Continued on next page

Detecting Fusion Proteins

Several antibodies are available from Invitrogen to detect expression of your fusion protein from pEF4/*myc*-His (see page 18).

To detect fusion protein by western blot, prepare a cell lysate from transfected cells. We recommend that you perform a time course to optimize expression of the fusion protein (*e.g.*, 24, 48, 72 hours, etc. after transfection). To lyse cells:

1. Wash cell monolayers (~10^6 cells) once with phosphate-buffered saline (PBS).
2. Scrape cells into 1 mL PBS and pellet the cells at 1,500 × *g* for 5 minutes.
3. Resuspend in 50 µL Cell Lysis Buffer (see recipe below). Other lysis buffers may be suitable.
4. Incubate cell suspension at 37°C for 10 minutes to lyse the cells.
5. Centrifuge the cell lysate at 10,000 × *g* for 10 minutes to pellet nuclei and transfer the supernatant to a fresh tube. Assay the lysate for protein concentration. **Note:** Do not use protein assays utilizing Coomassie Blue or other dyes. NP-40 interferes with the binding of the dye with the protein.
6. Add SDS-PAGE sample buffer to a final concentration of 1X and boil the sample for 5 minutes.
7. Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your fusion protein.

Note

The C-terminal peptide containing the *myc* epitope and the polyhistidine tag will add approximately 3 kDa to the size of your protein.

Purification

You will need lysate from 5 × 10^6 to 1 × 10^7 **transfected** cells for purification of your protein on a 2 mL ProBond™ column (or other metal-chelating column). Refer to the manufacturer's instructions before attempting to purify your fusion protein. To prepare cells for lysis, refer to the protocol on page 10.

Creating Stable Cell Lines

Selection in Mammalian Cell Lines

To generate a stable cell line expressing your protein, you need to determine the minimum concentration of Zeocin™ required for killing your untransfected host cell line. Typically, concentrations between 50 and 1000 µg/mL Zeocin™ are sufficient to kill the untransfected host cell line. Test a range of concentrations (see below) to ensure that you determine the minimum concentration necessary for your cell line.

1. Seed cells (2×10^5 cells/60 mm plate) for each time point and allow cells to adhere overnight.
2. The next day, substitute culture medium with medium containing varying concentrations of Zeocin™ (e.g., 0, 50, 125, 250, 500, 750, and 1,000 g/mL).
3. Replenish the selective medium every 3–4 days, and observe the percentage of surviving cells.
4. Count the number of viable cells at regular intervals to determine the appropriate concentration of Zeocin™ that prevents growth.

Possible Sites for Linearization

To obtain stable transfectants, you may choose to linearize your vector before transfection. While linearizing your vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts the gene of interest. The table below lists unique sites that may be used to linearize your construct prior to transformation. **Other restriction sites are possible. Note that for the enzymes listed below, the cleavage site is indicated for versions A, B, and C of pEF4/*myc*-His.** Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

Enzyme	Restriction Site (bp) (A,B,C)	Location	Supplier
Nru I	331	Upstream of EF-1α promoter	Many
Mlu I	351	Upstream of EF-1α promoter	Many
*Bst*1107 I	3688 (A), 3692 (B), 3684 (C)	End of SV40 poly A	AGS*, Fermentas, Takara, Boehringer-Mannhiem
*Eam*1105 I	4960 (A), 4964 (B), 4956 (C)	Ampicillin gene	AGS*, Fermentas, Takara
Fsp I	5182 (A), 5186 (B), 5178 (C)	Ampicillin gene	Many
Pvu I	5330 (A), 5334 (B), 5326 (C)	Ampicillin gene	Many
Sca I	5440 (A), 5444 (B), 5436 (C)	Ampicillin gene	Many

*Angewandte Gentechnologie Systeme

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Selecting Stable Integrants

Once the appropriate Zeocin™ concentration is determined, you can generate a stable cell line with your construct.

1. Transfect your cells using the appropriate protocol for your cell line. Include a sample of untransfected cells as a negative control.
2. After transfection, wash the cells once with 1X PBS and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing Zeocin™ at the appropriate concentration for your cell line. Split the cells such that they are no more than 25% confluent.
4. Change selective medium every 3–4 days until Zeocin™-resistant colonies are detected.
5. Pick and expand colonies.

Preparing Cells for Lysis

Use the procedure below to prepare cells for lysis prior to purification of your protein on ProBond™. You will need 5×10^6 to 1×10^7 cells for purification of your protein on a 2 mL ProBond™ column (see ProBond™ Protein Purification manual).

1. Seed cells in either five T-75 flasks or 2 to 3 T-175 flasks.
2. Grow the cells in selective medium until they are 80–90% confluent.
3. Harvest the cells by treating with trypsin-EDTA for 2 to 5 minutes or by scraping the cells in PBS.
4. Inactivate the trypsin by diluting with fresh medium (if necessary) and transfer the cells to a sterile microcentrifuge tube.
5. Centrifuge the cells at 240 *g* for 5 minutes. Resuspend the cells in PBS.
6. Centrifuge the cells at 240 *g* for 5 minutes. You may lyse the cells immediately or freeze in liquid nitrogen and store at –80°C until needed.

Lysis of Cells

If you are using ProBond™ resin, refer to the ProBond™ Protein Purification manual for details about sample preparation for chromatography.

If you are using other metal-chelating resin, refer to the manufacturer's instruction for recommendations on sample preparation.

Appendix

Human EF-1α Promoter

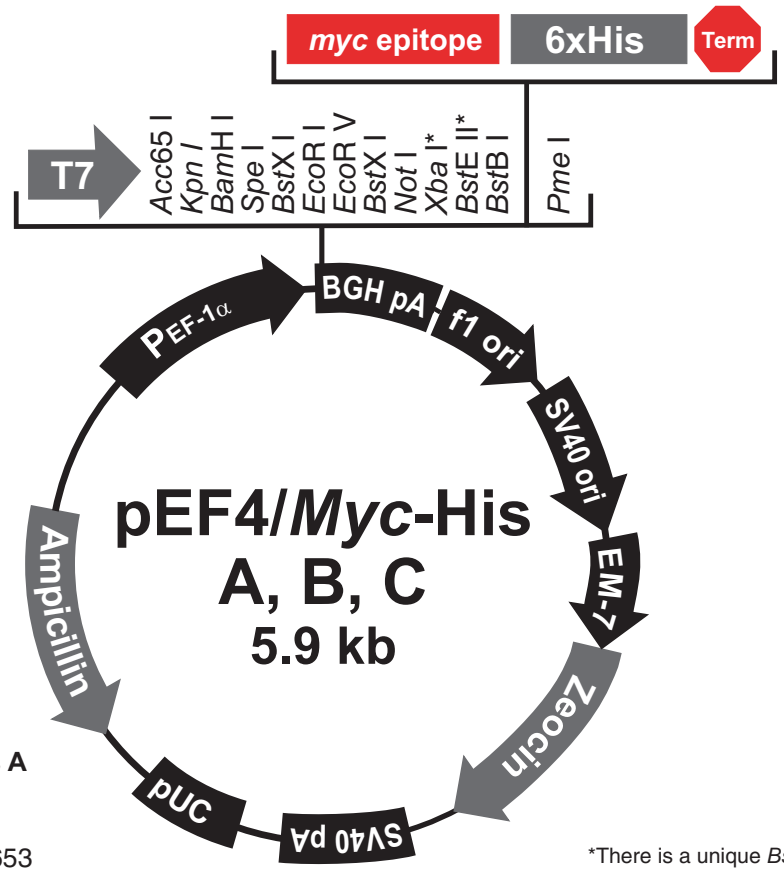
Description

The diagram below shows all the features of the EF-1α promoter used in the pEF4/*myc*-His vectors (Mizushima and Nagata, 1990). Features are marked as per Uetsuki *et al.*, 1989.

pEF4/*myc*-His Vector

Map of pEF4/*myc*-His

The figure below summarizes the features of the pEF4/*myc*-His vectors. The sequences for pEF4/*myc*-His A, B, and C are available for downloading from www.invitrogen.com or from **Technical Support** (see page 19).



*There is a unique *Bst*E II site, but no *Xba* I site in version C

Comments for pEF4/*Myc*-His A
5882 nucleotides

EF-1α promoter: bases 468-1653
T7 promoter/priming site: bases 1670-1689
Multiple cloning site: bases 1715-1806
myc epitope: bases 1804-1833
Polyhistidine tag: bases 1849-1866
BGH reverse priming site: bases 1889-1906
BGH polyadenylation signal: bases 1892-2119
f1 origin: bases 2165-2593
SV40 promoter and origin: bases 2621-2929
EM-7 promoter: bases 2977-3032
Zeocin™ resistance gene: bases 3051-3425
SV40 polyadenylation signal: bases 3555-3684
pUC origin: bases 4068-4741
Ampicillin resistance gene: bases 4886-5746

pEF4/*myc*-His Vector, Continued

Features of pEF4/*myc*-His

pEF4/*myc*-His A (5882 bp), pEF4/*myc*-His B (5886 bp), and pEF4/ *myc*-His C (5878 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human elongation factor 1α (hEF-1α) promoter	Allows overexpression of your recombinant protein in a broad range of mammalian cell types (Goldman *et al.*, 1996; Mizushima and Nagata, 1990).
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert.
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in frame with the *myc* epitope and C-terminal polyhistidine tag.
myc epitope (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of your recombinant protein with the Anti-*myc* Antibody or Anti-*myc*-HRP Antibody (Evans *et al.*, 1985).
C-terminal polyhistidine (6xHis) tag	Allows purification of your recombinant protein on metal-chelating resin such as ProBond™. In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His(C-term) Antibody (Lindner *et al.*, 1997) and the Anti-His (C-term)-HRP Antibody.
BGH reverse priming site	Allows sequencing through the insert.
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992).
f1 origin	Allows rescue of single-stranded DNA.
SV40 early promoter and origin	Allows efficient, high-level expression of the Zeocin™ resistance gene and episomal replication in cells expressing the SV40 large T antigen.
EM-7 promoter	Synthetic promoter based on the bacteriophage T7 promoter for expression of the Zeocin™ resistance gene in *E. coli.*
Zeocin™ resistance gene	Selection of transformants in *E. coli* and stable transfectants in mammalian cells (Drocourt *et al.*, 1990; Mulsant *et al.*, 1988).
SV40 polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA.
pUC-derived	High-copy number replication and growth in *E. coli.*
Ampicillin resistance gene (β-lactamase)	Selection of transformants in *E. coli.*

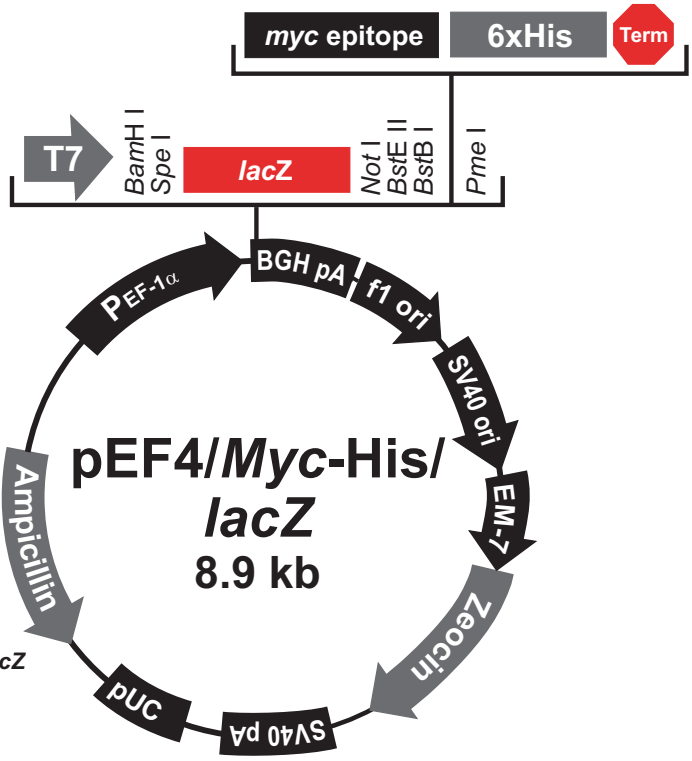
pEF4/*myc*-His/*lacZ*

Description

pEF4/*myc*-His/*lacZ* is a 8,932 bp control vector containing the gene for β–galactosidase. This vector was constructed by ligating a 3,976 bp *Bam*H I-*Bsm* I fragment containing the EF-1α promoter from pEF4/*myc*-His B to a 4956 bp *Bam*H I-*Bsm* I fragment containing the *lacZ* gene, *myc* epitope, polyhistidine tag and Zeocin™ resistance gene from pcDNA4/*myc*-His/*lacZ*.

Map of Control Vector

The figure below summarizes the features of the pEF4/*myc*-His/*lacZ* vector. The nucleotide sequence for pEF4/*myc*-His/*lacZ* is available for downloading from www.invitrogen.com or by contacting **Technical Support** (see page 19).



Comments for pEF4/*Myc*-His/*lacZ*
8932 nucleotides

EF-1α promoter: bases 468-1653
T7 promoter/priming site: bases 1670-1689
LacZ ORF: bases 1770-4826
myc epitope: bases 4851-4880
Polyhistidine tag: bases 4896-4913
BGH reverse priming site: bases 4936-4953
BGH polyadenylation signal: bases 4939-5166
f1 origin: bases 5212-5640
SV40 promoter and origin: bases 5668-5968
EM-7 promoter: bases 6027-6082
Zeocin™ resistance gene: bases 6101-6475
SV40 polyadenylation signal: bases 6605-6735
pUC origin: bases 7118-7791
Ampicillin resistance gene: bases 7936-8796

Zeocin™

Introduction

The pEF4/*myc*-His vectors contain the Zeocin™ resistance gene for selection of stable cell lines using Zeocin™. We recommend that you test the sensitivity of your mammalian host cell to Zeocin™ as natural resistance varies among cell lines. General information and guidelines are provided in this section for your convenience.

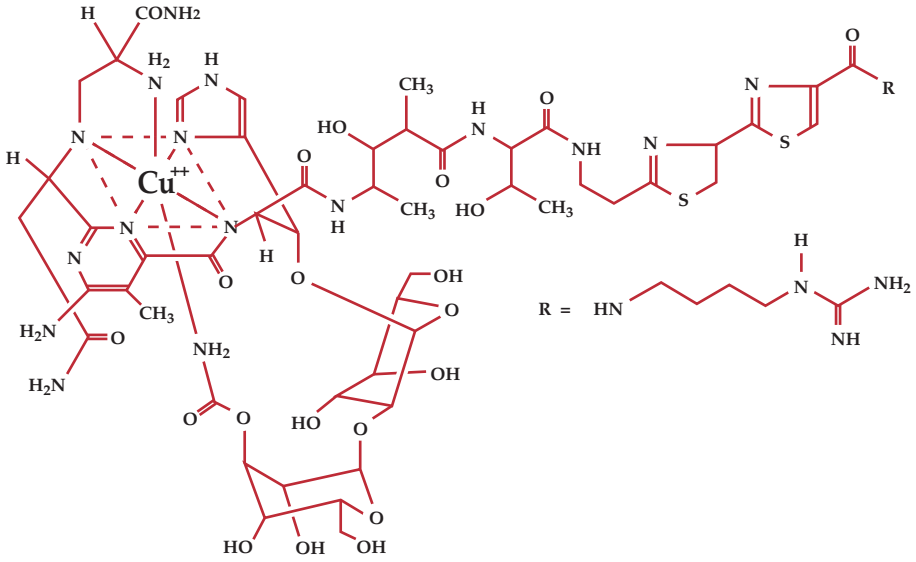
Zeocin™

Zeocin™ is a member of the bleomycin/phleomycin family of antibiotics isolated from *Streptomyces*. Antibiotics in this family are broad spectrum antibiotics that act as strong anti-bacterial and anti-tumor drugs. They show strong toxicity against bacteria, fungi (including yeast), plants, and mammalian cells (Baron *et al.*, 1992; Drocourt *et al.*, 1990; Mulsant *et al.*, 1988; Perez *et al.*, 1989).

The Zeocin™ resistance protein has been isolated and characterized (Calmels *et al.*, 1991; Drocourt *et al.*, 1990). This protein, the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), is a 13.7 kDa protein that binds Zeocin™ and inhibits its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™.

Molecular Weight, Formula and Structure

The formula for Zeocin™ is $C_{55}H_{86}O_{21}N_{20}S_2Cu.HCl$ and the molecular weight is 1,527.5 Da. Zeocin is an HCl salt. The diagram below shows the structure of Zeocin™.



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Zeocin™, Continued

Applications of Zeocin™

Zeocin™ is used for selection in mammalian cells (Mulsant *et al.*, 1988); plants (Perez *et al.*, 1989); yeast (Baron *et al.*, 1992); and prokaryotes (Drocourt *et al.*, 1990). Suggested concentrations of Zeocin™ for selection in mammalian cell lines and *E. coli* are listed below:

Organism	Zeocin™ Concentration and Selective Medium
E. coli	25–50 µg/mL in low salt LB medium* (see page 17 for recipe)
Mammalian Cells	50–1000 µg/mL (varies with cell line)

*Efficient selection requires that the concentration of NaCl be no more than 5 g/liter (<90 mM).

Handling Zeocin™

- High salt and acidity or basicity inactivates Zeocin™. Therefore, we recommend that you reduce the salt in bacterial medium and adjust the pH to 7.5 to keep the drug active (see page 17).
- Store Zeocin™ at –20°C and thaw on ice before use.
- Zeocin™ is light sensitive. Store drug, plates, and medium containing drug in the dark.
- Wear gloves, a laboratory coat, and safety glasses or goggles when handling solutions containing Zeocin™.
- Zeocin™ is toxic. Do not ingest or inhale solutions containing the drug.

Recipes

Low Salt LB Medium with Zeocin™

For Zeocin™ to be active, the salt concentration of the medium must be low (<90 mM) and the pH must be 7.5. For selection in *E. coli*, it is **imperative** that you prepare LB broth and plates using the following recipe. Note the lower salt content of this medium. Failure to use low salt LB medium will result in non-selection due to inactivation of the drug.

Low Salt LB Medium:

10 g Tryptone
5 g NaCl
5 g Yeast Extract

1. Combine the dry reagents above and add deionized, distilled water to 950 mL. Adjust pH to 7.5 with 5 M NaOH. Bring the volume up to 1 liter. For plates, add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle at 15 lbs/sq. in. and 121°C for 20 minutes.
3. Thaw Zeocin™ on ice and vortex before removing an aliquot.
4. Allow the medium to cool to at least 55°C before adding the Zeocin™ to 25 µg/mL final concentration.
5. Store plates at 4°C in the dark. Plates containing Zeocin™ are stable for 1-2 weeks.

Cell Lysis Buffer

50 mM Tris-HCl, pH 7.8
150 mM NaCl
1% Nonidet P-40

1. This solution can be prepared from the following common stock solutions. For 100 mL, combine:

1 M Tris base	5 mL
5 M NaCl	3 mL
Nonidet P-40	1 mL

2. Bring the volume up to 90 mL with deionized water and adjust the pH to 7.8 with HCl.
3. Bring the volume up to 100 mL. Store at room temperature.

Note: Protease inhibitors may be added at the following concentrations:

1 mM PMSF
1 µg/mL pepstatin
1 µg/mL leupeptin

Accessory Products

Introduction

The following products may be used with the pEF4/*myc*-His vectors. For details, visit www.invitrogen.com or contact **Technical Support** (page 19).

Item	Amount	Catalog no.
ProBond™ Purification System	6 × 2 mL precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification	K850-01
ProBond™ Resin	50 mL	R801-01
	150 mL	R801-15
Anti-Xpress™ Antibody		R910-25
Electrocomp™ TOP10F´	5 × 80 µL	C665-55
One Shot® TOP10F´ Chemically Competent *E. coli*	20 × 50 µL	C3030-03
PureLink™ HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
β-Gal Assay Kit	80 mL	K1455-01
β-Gal Staining Kit	1 kit	K1465-01
Zeocin™	1 gram	R250-01
	5 gram	R250-05
Lipofectamine™ 2000 Reagent	0.75 mL	11668-027

Primers

For your convenience, Invitrogen offers a custom primer synthesis service. Visit www.invitrogen.com for more details.

Antibodies

If you do not have an antibody specific to your protein, Invitrogen offers the Anti-*myc*, or Anti-His(C-term) antibodies to detect your recombinant fusion protein. Horseradish peroxidase (HRP)- and alkaline phosphatase (AP)–conjugated antibodies are available for convenient one-step detection.

Antibody	Epitope	Catalog no.
Anti-*myc*	Detects a 10 amino acid epitope derived from *c-myc* (Evan *et al.*, 1985): EQKLISEEDL	R950-25
Anti-*myc*-HRP		R951-25
Anti-*myc*-AP		R952-25
Anti-His(C-term)	Detects the C-terminal polyhistidine tag (requires the free carboxyl group for detection) (Lindner et al., 1997): HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP		R931-25
Anti-His(C-term)-AP		R932-25

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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SDS

Safety Data Sheets (SDSs) are available on our website at www.invitrogen.com/sds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

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